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(21) International Application Number: PCT/AU99/00166 (22) International Filing Date: 16 March 1999 (16.03.99) (30) Priority Data: PP 2503 20 March 1998 (20.03.98) AU (71) Applicant (for all designated States except US): USF FILTRATION AND SEPARATIONS GROUP INC. [US/US]; 2118 Greenspring Drive, Timonium, MD 21093 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): HODGES, Alastair, McIndoe [AU/AU]; 15 Jasmine Court, Blackburn South, VIC 3130 (AU). CHATELIER, Ronald, Christopher [AU/AU]; 9 Apple Grove, Bayswater, VIC 3153 (AU). (74) Agent: BALDWIN SHELSTON WATERS; 60 Margaret Street, Sydney, NSW 2000 (AU).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: SENSOR WITH IMPROVED SHELF LIFE		
(57) Abstract		
<p>The present invention provides a metal electrode stabilised by a coating, the coating comprising a sulfur containing moiety in its molecular structure. The coating may also include a hydrophilic group and a spacer between the sulfur containing moiety and the hydrophilic group. Preferably, the sulphur-containing moiety is selected from the group comprising thiol, disulphide and SO_x, and the hydrophilic group is selected from the group comprising hydroxyl, amine, carboxyl, carbonyl, oligo (ethylene oxide) chain, and zwitterionic species. Compounds useful in the present invention include 2-mercaptoethanol, 2-mercaptoethylamine, 3-mercaptopropionic acid, thiophene, 4-carboxythiophene, cysteine, homocysteine, and cystine.</p>		

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SENSOR WITH IMPROVED SHELF LIFE

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TECHNICAL FIELD

The invention relates to apparatus comprising one or more metal electrodes such as electrochemical cells, sensor elements and the like, and more particularly to extending the shelf life of such apparatus.

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BACKGROUND ART

Metal electrodes have proved useful in sensor elements for sensing a diverse range of biologically important molecules eg glucose, and for determining physical properties such as pH. A range of possible configurations and applications involving metal electrodes are discussed in our co-pending applications PCT/AU96/00210, PCT/AU96/00365 and PCT/AU96/00723.

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A desirable attribute of all sensor elements is that they have a long shelf life - that is, the sensing characteristic of the sensor element does not change significantly between manufacture and use (ie on storage).

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In an electrochemical sensor element the stability of the electrode is critical to the stability of the sensor as a whole. Typically, when left to stand for long periods of time, electrodes become prone to instability in subsequent use thus limiting the useful shelf life. It is thought that such instability is caused by absorption or reaction of the metallic surface with atmospheric contaminants. It has also been observed that filling time of sensors deteriorates on prolonged storage.

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It is an object of the present invention to overcome or ameliorate at least some of the above disadvantages in the prior art.

Surprisingly, the present applicant has found that by coating a metal electrode with a monolayer or multilayer of selected materials, electrode behaviour can be significantly stabilised in comparison with uncoated metal electrodes without loss of the desirable sensing characteristics of the electrodes.

30

DESCRIPTION OF THE INVENTION

According to a first aspect, the invention consists in a metal electrode stabilised by a coating, the coating comprising a sulphur-containing moiety in its molecular structure.

“Comprising” as herein used is used in an inclusive sense, that is to say in the sense of but not limited to “including” or “containing”. The term is not intended in an exclusive sense (“consisting of” or “composed of”).

Preferably, the sulphur-containing moiety is selected from the group comprising
5 thiol, disulphide and SO_x . Most preferably the sulphur-containing moiety is a disulphide. The sulphur-containing moiety may also be incorporated in a cyclic structure.

According to a second aspect, the invention consists in a metal electrode stabilised by a coating according to the first aspect, further comprising a hydrophilic group in its molecular structure.

10 Preferably, the hydrophilic group is selected from the group comprising hydroxyl, amine, carboxyl, carbonyl, oligo (ethylene oxide) chain, and zwitterionic species. Most preferably, the hydrophilic group is a zwitterionic species. The most preferred zwitterionic species comprises an amine and a carboxyl group in proximity.

According to a third aspect, the invention consists in a metal electrode stabilised by a
15 coating according to the second aspect, further comprising a spacer between the sulphur-containing moiety and the hydrophilic group.

Preferably, in the third aspect, the spacer consists of an alkyl group or an aromatic group. It is preferable that methylene or ethylene groups be included in the spacer element.

According to a fourth aspect, the invention consists in a method of preparing a metal
20 electrode stabilised by a coating, comprising the step of contacting a metal electrode with a substance comprising a sulphur-containing moiety in its molecular structure.

According to a fifth aspect, the invention consists in a method of preparing a metal electrode stabilised by a coating, comprising the steps of contacting a metal electrode with a substance comprising a sulphur-containing moiety and a hydrophilic group in its
25 molecular structure.

According to a sixth aspect, the invention consists in a method of preparing a metal electrode stabilised by a coating comprising the steps of contacting a metal electrode with a substance comprising a sulphur-containing moiety, a hydrophilic group and a spacer between the sulphur-containing moiety and the hydrophilic group in its molecular
30 structure.

The preferred substances for use in the methods described in the fourth, fifth and sixth aspects are identical to those substances described in respect of the first, second and third aspects.

The invention also consists in a method of sensing an analyte, comprising the step of substituting the electrode in a known sensor device with a metal electrode stabilised by a coating according to the present invention, and sensing an analyte.

BEST MODE FOR CARRYING OUT THE INVENTION

Various embodiments of the invention will now be described by way of example only.

It is known in the prior art that thiols form coatings on metals. Thiols have also been used to tether species such as antibodies onto metal surfaces, for instance those of gold particles, for the purposes of immobilisation etc. One would expect that such coatings would also bind contaminants to the surface.

As much electrode chemistry involves interaction at the electrode surface, it is thus surprising that coatings used to bind molecules to the metal surface can be useful in preventing contamination of the electrode surface. It is also surprising that notwithstanding the application of the coating an electrode retains desirable electrochemical properties. The procedure for preparing the metal electrode stabilised by a coating involves contacting a metal electrode with selected sulphur-containing compounds, such as thiols, disulphides and compounds of the formula SO_x among others being suitable in the context of the present invention. The coatings also desirably contain a hydrophilic group which includes such species as hydroxyl, amino, carboxyl, carbonyl, oligo (ethylene oxide) chains and zwitterionic species. The latter two compounds indicate that compounds having one or more hydrophilic groups are also suitable groups for use in the present invention.

Between the sulphur group, which acts to tether the molecule onto the metal surface, and the hydrophilic group, which presents a hydrophilic surface, spacers may be employed.

Compounds useful in the present invention include, but are not limited to 2-mercapto ethanol, 2-mercaptoethylamine, 3-mercaptopropionic acid, thiophene, 4-carboxy thiophene, cysteine, homocysteine and cystine. Most preferably the molecule is cystine. In any of the above aspects, the D or L isomers can be used or mixtures of D and L isomers can be used, where such isomers are possible.

The compound in accordance with the invention is then applied as a monolayer or multilayer onto the surface of the electrode. It is possible to apply the compound by simply exposing the electrode to the coating material, with the coating material in either the vapour phase or in solution. The substance can be applied by dipping, spraying, painting, printing etc. After application, it is possible to wash the surface of the contacted electrode.

In a further aspect of the current invention the layer of the sulfur containing compound can optionally be overcoated with a surfactant layer. The surfactant layer can be applied after the application of the sulfur containing layer or at the same time as the sulfur containing layer, for example the sulfur containing species and the surfactant can be placed in a coating bath into which the electrode material is immersed. Due to the higher affinity of the sulfur containing species for the electrode material it will bind to the electrode surface in preference to the surfactant, leaving the surfactant in a layer over the sulfur containing layer. An example of a suitable surfactant is Triton X-100.

15 EXAMPLES

EXAMPLE 1 - PREPARATION

The electrode coatings were applied to gold or palladium electrodes by immersing the sheet of material from which the electrodes were made into a 1 mM aqueous solution of the coating compound adjusted to pH 12 by the addition of potassium hydroxide. The contact time between the electrode material and the coating bath was typically 30 seconds. After coating, the electrodes were washed by immersion in a bath of water. In some cases, the electrodes were immersed in a third bath containing 1,000 ppm of triton X-100 in water. Finally, the electrode material sheets were dried by blowing with air at room temperature.

25 EXAMPLE 2 - STORAGE

The data in Tables 1 and 2 below show the effect on the electrode stability of coating the electrodes with sulphur-containing compounds. The stability was assessed using an accelerated test. The glucose sensors using coated or uncoated electrodes were stored either at 4°C in the refrigerator ("fridge") or at 56°C in an oven for two weeks. The sensors stored at 4°C do not change significantly from their performance when freshly prepared and tested. Those stored in the oven are subject to accelerated ageing, which simulates longer ageing times at room temperature.

EXAMPLE 3 - TESTING

After two weeks the sensors were tested with whole blood samples with various glucose concentrations, from about 3 mM to 30 mM. The background ferrocyanide concentration was measured (the reading obtained when a sample contains no glucose) and the overall precision and fill speed of the sensors was assessed. The effect of the electrode coatings is shown in Table 1. The fill speeds in Table 1 were assessed qualitatively by eye. The fill speeds in Table 2 were assessed quantitatively by videoing the filling of the sensor with a blood sample using an on-screen timer and subsequently determining the number of seconds required for the blood to fill each sensor.

It can be seen from the first pair of results, for a non-coated electrode, that artificial ageing dramatically increased the %cv (corresponding to decreased precision).

In contrast, for the last two pairs of results, the %cv's for the treated electrodes after artificial ageing were comparable to the %cv's of untreated electrodes on fridge storage and significantly better than accelerated aged untreated electrodes.

A desirable side effect of the present invention also appears to be maintenance of good fill speed for sensors on ageing.

TABLE 1
TEST DATA

STORAGE	COATING	BACKGROUND (mM ferrocyanide)	MEAN %cv	FILL SPEED
Fridge	None	1.01	3.8	OK
Oven	None	5.12	10.05	very slow
Fridge	Cysteine	1.3	4.5	OK
Oven	Cysteine	5.0	8.0	slow
Fridge	Cysteine/trit	1.98	3.1	fast
Oven	Cysteine/trit	2.17	5.4	OK
Fridge	Homocysteine /trit	1.02	4.6	OK
Oven	Homocysteine /trit	2.34	4.2	faster than Cysteine/trit
Fridge	Cystine/trit	0.63*	4.1	fast
Oven	Cystine/trit	1.24*	4.4	good

* saline rather than blood used to assess the background

Trit denotes an overcoating of Triton X-100.

TABLE 2

PRECISE FILL TIMES

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STORAGE	COATING	FILL TIME (secs)
Fridge	none	1.0
Oven	none	5.3
Fridge	Cystine	0.4
Oven	Cystine	4.0
Fridge	Cystine/trit	0.3
Oven	Cystine/trit	1.4

A person skilled in the art will appreciate that the application process is very simple and facile and could be accomplished from the teaching hereof in many ways.

THE CLAIMS OF THE INVENTION ARE AS FOLLOWS

1. A metal electrode stabilised by a coating, the coating comprising a sulfur containing moiety in its molecular structure.
- 5 2. A metal electrode according to Claim 1 wherein the sulfur containing moiety is selected from the group consisting of thiol, disulfide and SO_x .
3. A metal electrode according to Claim 2 wherein the sulfur containing moiety is a disulfide.
4. A method according to any one of the preceding claims wherein the sulfur containing
10 moiety is further incorporated in a cyclic structure.
5. A metal electrode according to any one of claims 1 to 4 stabilised by a coating, said coating comprising a sulfur containing moiety and a hydrophilic group in its molecular structure.
6. A metal electrode according to Claim 5 wherein the hydrophilic group is selected
15 from the group consisting of hydroxyl, amine, carboxyl, carbonyl, oligo (ethyleneoxide) chain, and zwitterionic species.
7. A metal electrode according to Claim 6 wherein the hydrophilic group is a zwitterionic species.
8. A metal electrode according to Claim 7 wherein the zwitterionic species includes an
20 amine and a carboxyl group in proximity.
9. A metal electrode according to any one of the preceding claims stabilised by a coating, said coating comprising a sulfur containing moiety, a hydrophilic group, and a spacer between the sulfur containing moiety and the hydrophilic group in its molecular structure.
- 25 10. A metal electrode according to Claim 9 wherein the spacer is an alkyl group or an aromatic group.
11. A metal electrode according to Claim 10 wherein the alkyl group includes methylene and/or ethylene groups as part of the spacer element.
12. A metal electrode according to any one of the preceding claims wherein the coating
30 is selected from the group consisting of 2-mercaptoethanol, 2-mercaptoethylamine, 3 mercaptopropionic acid, thiophen, 4-carboxythiophen, cysteine, homocysteine, and cystine.

13. A metal electrode according to any one of the preceding claims wherein the compound is the D or L isomer.
14. A metal electrode according to any one of claims 1 to 12 wherein the compound is a mixture of D and L isomers.
- 5 15. A metal electrode according to any one of the preceding claims further including an overcoating of surfactant.
16. A method of preparing a metal electrode stabilised by a coating, including the step of contacting a metal electrode with a substance, said substance including a sulfur containing moiety in its molecular structure.
- 10 17. A method according to claim 16 comprising the step of contacting a metal electrode with a substance including a sulfur containing moiety and a hydrophilic group in its molecular structure.
18. A method according to claim 16 or 17, comprising the step of contacting a metal electrode with a substance comprising a sulfur containing moiety, a hydrophilic group and
15 a spacer between the sulfur containing moiety and the hydrophilic group in its molecular structure.
19. A method according to any one of claims 16 to 18 wherein the coating includes compounds selected from the group consisting of 2-mercaptoethanol, 2-mercaptoethylamine, 3-mercaptopropionic acid, thiophen, 4-carboxythiophen, cysteine,
20 homocysteine, and cystine.
20. A method according to any one of claims 16 to 19 wherein the compound is the D or L isomer.
21. A method according to any one of claims 16 to 19 wherein the compound is a mixture of D and L isomers.
- 25 22. A method according to any one of claims 16 to 21 wherein the coating is applied as a monolayer or multilayer on the surface of an electrode.
23. A method according to any one of claims 16 to 22 wherein the coating material is applied by deposition from the vapour phase or from solution.
24. A method according to any one of claims 16 to 23 wherein the substance is applied
30 by a method selected from the group consisting of dipping, spraying, painting, and printing.

25. A method according to any one of claims 16 to 24 further including the step of overcoating the substance including a sulfur containing moiety in its molecular structure with a surfactant layer.
26. A method according to claim 25 wherein the surfactant layer and sulfur containing
5 layer are applied simultaneously.
27. A method according to claim 25 wherein the surfactant layer is applied to the electrode subsequent to the application of the coating compound.
28. A method of sensing an analyte including the step of substituting the electrode in a known sensor device with a metal electrode stabilised by a coating according to any one of
10 the preceding claims, and sensing an analyte.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00166

A. CLASSIFICATION OF SUBJECT MATTERInt Cl⁶: G01N 27/30, 27/403; C23C 22/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC : G01N; C23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

IPC : AU as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPAT, JAPIO, CHEM ABS: Electrode, gold, metal, palladium, coal, layer, sulph, thio-, disul-, cysteine, cystine, hydrophilic, zwitter-, mercapto-, methion:

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<u>Analytica Chimica Acta</u> vol. 364 no. 1-3 (1998) "Glucose oxidase/polyion complex-bilayer membrane for elimination of electroactive interferents in amperometric glucose sensor" Mizutani, F. et al., pages 173-179 abstract	1,2,5,6,9-28
X	<u>Talanta</u> vol 44 no. 5 (1997) "Self-assembled biotinylated disulfide derivative monolayer on gold electrode for immobilising enzymes" Pingang, H. et al., pages 885-890 Page 886 "2.2 Electrode preparation"	1-3,5,6,9-14,16-24,28
X	<u>Bioelectrochemistry and Bioenergetics</u> vol 42 no. 1 (1997) "Self assembled monolayers of thiols on gold electrodes for bioelectrochemistry and biosensors" Dong, S. et al., pages 7-13 abstract	1,2,5,6,9-14,16-24,28

☒ Further documents are listed in the
continuation of Box C

☒ See patent family annex

* Special categories of cited documents:

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PCT/AU 99/00166

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<u>Chemistry Letters</u> vol 12 (1993) "Glucose sensitivity of Thiol-modified Gold Electrodes Having Immobilised Glucose Oxidase and 2-Aminoethylferrocene" Kajiya, Y et al. abstract	1,2,4,6,9- 10,16-18,22- 24,28
X	WO 97/43274 (AUSTRALIAN MEMBRANE AND BIOTECHNOLOGY RESEARCH INSTITUTE) 20 November 1997 page 2 line 11 - page 6 line 25	1-6,9-14,16- 24,28
X	WO 91/09304 (UNITED STATES DEPARTMENT OF ENERGY) 27 June 1991 page 6 lines 13-33	1,2,16,22- 24,28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00166

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	97/43274	AU	26283/97	CA	2254072		
WO	91/09304	CA	2045616	DE	69025470	EP	457892
		JP	4505966	US	5082550		

END OF ANNEX